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GB 2124224 A EP 0128670 A US 4672057 A

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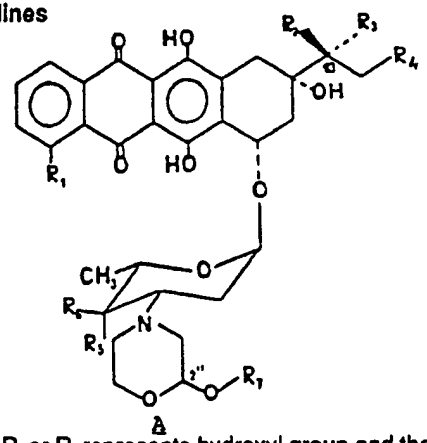
(54) **13-dihydro-3'-(2-alkoxy-4-morpholinyl)anthracyclines**

(57) Anthracycline glycosides of formula *A*:



A

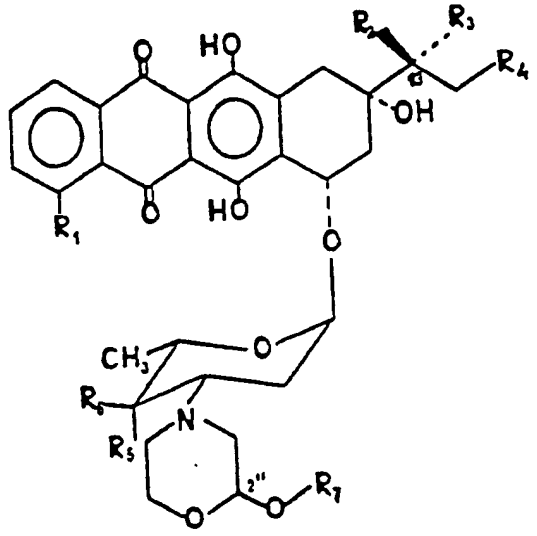
wherein R_1 is hydrogen atom, hydroxy or methoxy group; R_2 or R_3 represents hydroxyl group and the other of R_2 and R_3 represents hydrogen atom; R_4 is hydrogen or hydroxy; both R_5 and R_6 represent hydrogen or one of R_5 and R_6 represents hydrogen and the other of R_5 and R_6 hydroxy; R_7 represents a lower linear or branched alkyl residue or benzyl residue and their pharmaceutically acceptable salts are *anti-tumour agents.*

GB 2 247 3

13-DIHYDRO-3'-(2-ALKOXY-4-MORPHOLINYL)ANTHRACYCLINES

The invention relates to new anthracycline glycosides, to processes for their preparation and to pharmaceutical compositions containing them.

The invention provides new anthracycline glycosides of general formula A:



A

wherein R_1 is a hydrogen atom, hydroxy or methoxy group; one of R_2 and R_3 represents a hydroxyl group and the other of R_2 and R_3 represents hydrogen; R_4 is hydrogen or hydroxy; both R_5 and R_6 represent hydrogen or one of R_5 and R_6 is hydroxy and the other of R_5 and R_6 is hydrogen; R_7 represents a lower linear or branched alkyl, preferably containing from 1 to 10 carbon atoms, or benzyl group; or a pharmaceutically acceptable salt thereof.

Thus the compounds of the invention are 13-dihydro-anthracycline glycosides in which a 3'-nitrogen atom is

enclosed in a 2-alkoxy-4-morpholino ring.

The alkyl group represented by R_7 may for example contain from 1 to 6, preferably 1 to 4, carbon atoms and may for instance be methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl or tert-butyl.

The compounds may be in the form of a mixture of isomers in which the 13-carbon atom has an S or an R configuration, such as the racemate.

Alternatively the compounds may be in optically pure form. The compounds may be in the S configuration at the 13-carbon atom and be substantially free of the isomer with the R configuration at the 13-carbon atom, or they may be in the R configuration at the 13-carbon atom and be substantially free of the isomer with the S configuration at the 13-carbon atom.

Particularly preferred compounds are those in which the 13-carbon atom has an S-configuration, i.e. wherein R_2 = OH and R_3 = H. Preferred pharmaceutically acceptable salts are acid addition salts such as hydrochlorides. Preferred embodiments of the new anthracycline glycosides of general formula A include:

A1: 13-(R/S)-dihydro-3'-deamino-3'-(2-methoxy-4-morpholinyl)-doxorubicin
(R_1=OCH_3, R_2=OH/H and R_3=H/OH, R_4=R_5=OH, R_6=H, R_7=CH_3)

A2: 13-(S)-dihydro-3'-deamino-3'-(2-methoxy-4-morpholinyl) doxorubicin

A3: 13-(S/R)-dihydro-4'epi-3'-deamino-3'-(2-methoxy-4-morpholinyl)-doxorubicin

(R_1=OCH_3, R_2=OH/H and R_3=H/OH, R_4=R_6=OH, R_5=H, R_7=CH_3)

A4: 13-(S)-dihydro-4'epi-3'-deamino-3'-(2-methoxy-4-morpholinyl)-doxorubicin

(R_1=OCH_3, R_2=OH and R_3=H, R_4=R_6=OH, R_5=H, R_7=CH_3)

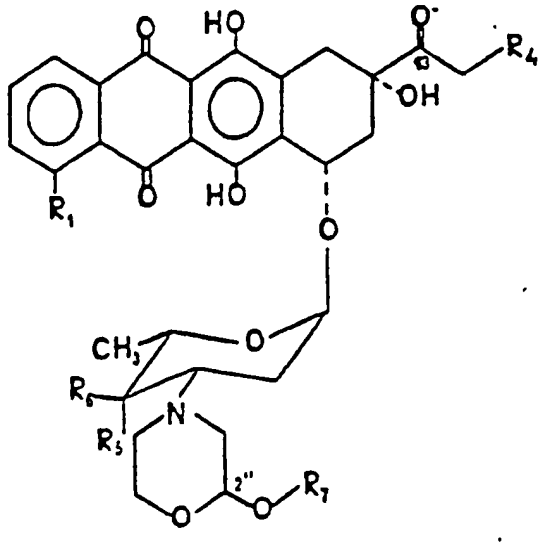
A5: 13-(S/R)-dihydro-4-demethoxy-3'-deamino-3'-(2-methoxy-4-morpholinyl)daunorubicin

(R_1=R_4=R_6=H, R_2=OH/H and R_3=H/OH, R_5=OH, R_7=CH_3)

A6: 13-(S)-dihydro-4-demethoxy-3'-deamino-3'-(2-methoxy-4-morpholinyl)daunorubicin

(R_1=R_4=R_6=H, R_2=OH and R_3=H, R_5=OH, R_7=CH_3)

The dihydro-anthracyclines of the present invention may be prepared by several methods. The present invention, provides a first process for preparing a compound of formula A or a pharmaceutically acceptable salt thereof which first process comprises reducing the 13-carbonyl group of a compound of general formula B:



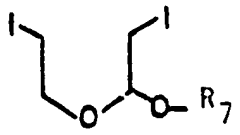
wherein R_1, R_4, R_5, R_6 and R_7 have the same meaning as above defined or a salt thereof, such as a pharmaceutically acceptable salt, eg the hydrochloride addition salt and, if desired, converting the resulting compound of formula A into a pharmaceutically acceptable salt.

The reduction may be effected using sodium borohydride in organic solvents such as methanol or using sodium cyanoborohydride for example in a mixture of acetonitrile and water typically at a pH from 7 to 4. Preferably the reaction is carried out at 0°C for 5 minutes. This process affords a 1:1 mixture of 13(R)- and 13(S)-dihydro anthracyclines (Scheme 1). If the product is in the form of a free base it is preferably treated with methanolic hydrogen chloride and isolated as its hydrochloride. The starting compounds of formula B are described US-A-4,672,057 and our copending GB Patent Application No. 9007513.6, or in E.W. Acton in Bioactive Molecules, 55-101, vol 6, Edited by J.W. Lown, Elsevier 1988 and may be prepared by the methods described therein.

In particular GB Application No. 9007513.6 discloses compounds of formula B, in which R_1 is OMe, R_4 is OH, R_5 is OH, R_6 is H and R_7 is C_2-C_6 alkyl and their pharmaceutically acceptable salts. It discloses that there may be prepared by

(i) reacting doxorubicin or an acid addition salt thereof, for example the hydrochloride salt, with a

diiodo compound of general formula C:



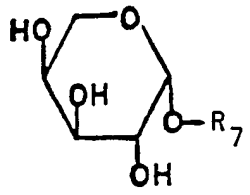
wherein R_7 is as defined above; and

(ii) if desired, converting the anthracycline glycoside of formula (A) thus obtained into a pharmaceutically acceptable acid addition salt thereof. It will be apparent to the person skilled in the art that analogous processes may be used to prepare other compounds of formula B and their salts.

The alkylation of the C-3' amino group of doxorubicin or the doxorubicin salt is typically performed in step (i) in a polar aprotic solvent and in the presence of a dry organic base such as triethylamine. Reaction is generally carried out at room temperature from eight to twenty four hours. The carbon atom C-2 bearing the $-OR_7$ group in the diiodo compound may have a (S) or (R) configuration. In a preferred embodiment, doxorubicin or its hydrochloride, dissolved in a polar aprotic solvent is reacted at room temperature and in the presence of a dry organic base, with the diiodo compound of general formula C to give the corresponding morpholinyl doxorubicin derivative of formula B which, after purification on a silica gel

column using as eluting system methylene chloride-methanol (97:5 v/v), is isolated, by treatment with methanolic anhydrous hydrogen chloride, as its hydrochloride.

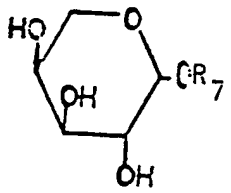
The optically pure diiodo compounds C may be prepared starting from sugar precursors such as the compounds of general formula S derived from L-arabinose:



S

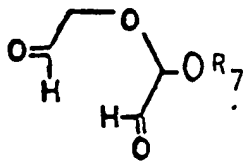
wherein R_7 is as defined above. This process comprises:

(a) subjecting to periodate oxidation a compound of formula S^1:



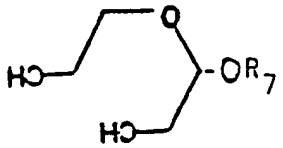
wherein R_7 is as defined above;

(b) reducing the thus-obtained dialdehyde derivative of formula T':



wherein R_7 is as defined above;

(c) sulfonating the thus-obtained dihydroxy derivative of formula U^1:



wherein R_7 is a defined above; and

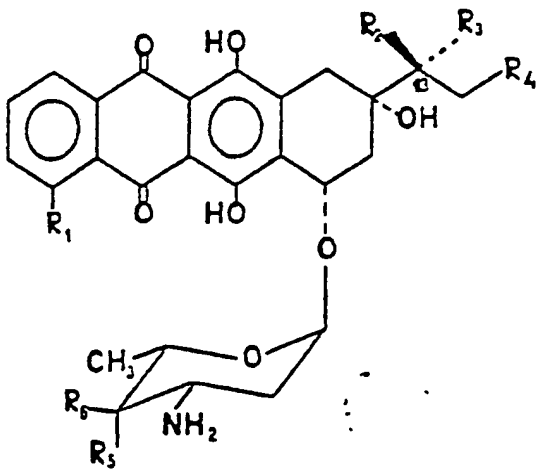
(d) iodinating the sulfonated derivative thus obtained.

In order to prepare the diiodo compounds C, 1-substituted sugars S^1, prepared following standard procedures described in "Methods on Carbohydrate Chemistry" Acad. Press., Vol 1, (1962), are first transformed into dialdehyde derivatives T^1. Generally, D- or L-arabinose is employed as a starting material. This is reacted with an alcohol R_7-OH thereby to form the compound of formula S^1. The dialdehyde derivatives can be obtained by using periodate oxidation in water, then reduced to 1,5-dihydroxy-2-alkoxy or -benzyloxy-3-oxa-pentane U^1 by using reducing agents such as sodium borohydride or sodium cyanoborohydride at pH 6.5 in a mixture of water and methanol.

The resultant dihydro compounds U^1 are sulfonated at the 1- and 5-hydroxyl groups, typically by using p-toluensulfonyl chloride in pyridine at 4°C to give the

sulfonyl ester of from which the diiodo derivatives C are obtained upon treatment with sodium or potassium iodide in aprotic solvent such as methylethylketone at 85°C from one to two days. The sequence of these reactions do not affect the chirality at C-2 of the diiodo derivatives C which is the same as the starting sugars S.

Other methods allow the preparation of optically pure 13(S)-dihydro anthracyclines of general formula A (R_2=OH and R_3=H). Therefore the invention further provides a second process for the preparation of a compound of formula A, or a pharmaceutically acceptable salt thereof, which process comprises reacting an optically pure 13(S)-dihydro-anthracycline of general formula D:



D

wherein R_1, R_4, R_5 and R_6 have the same meaning as above defined, R_2 represents hydroxyl group and R_3 is hydrogen or a salt thereof, such as a pharmaceutically acceptable salt e.g. the hydrochloride addition salt with (a) diiodo or (b)

dialdehyde derivatives of general formula E:

$$X\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH(OR_7)\text{-}CH_2\text{-}X$$

E

wherein X represent iodine atom or formyl group (-CHO) and R_7 has the same meaning as above defined and, if desired, converting the resulting compound of formula A into to a pharmaceutically acceptable salt.

More particularly, compounds of general formula D may be alkylated (a) by using diiodo derivatives of formula E (X=I) in the manner described in GB Patent Application No. 9007513.6, in dry polar and aprotic solvents such as acetonitrile or dimethylformamide in the presence of a dry organic base, such as triethylamine typically at room temperature from 4 to 24 hours (Scheme 2), or may be reductively alkylated (b) using dialdehyde derivatives of formula E (X=CHO) in aqueous media typically at pH from 5 to 4 in the presence of a reducing agent such as sodium cyanoborohydride (Scheme 3).

If the product of either of these reactions is in the form of a free base it is preferably treated with methanolic hydrogen chloride and isolated as its hydrochloride.

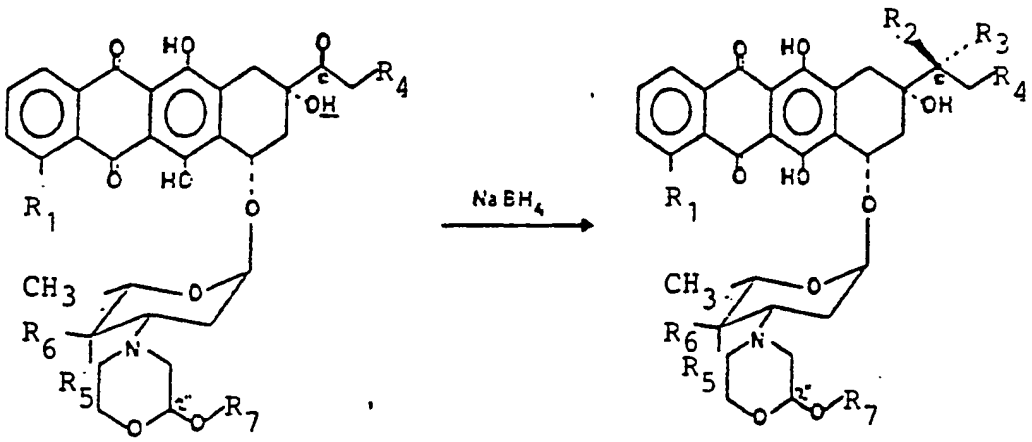
The compounds of formula D may be prepared by as disclosed in US-A-4,438,105.

It will be appreciated that the compounds of formula E in which X is I correspond to the compounds of formula C described above and the compounds may be prepared

as described above. The compounds of formula E in which X is CHO may be prepared as disclosed in US-A-4,672,057 or by analogous methods which would be apparent to the person skilled in the art.

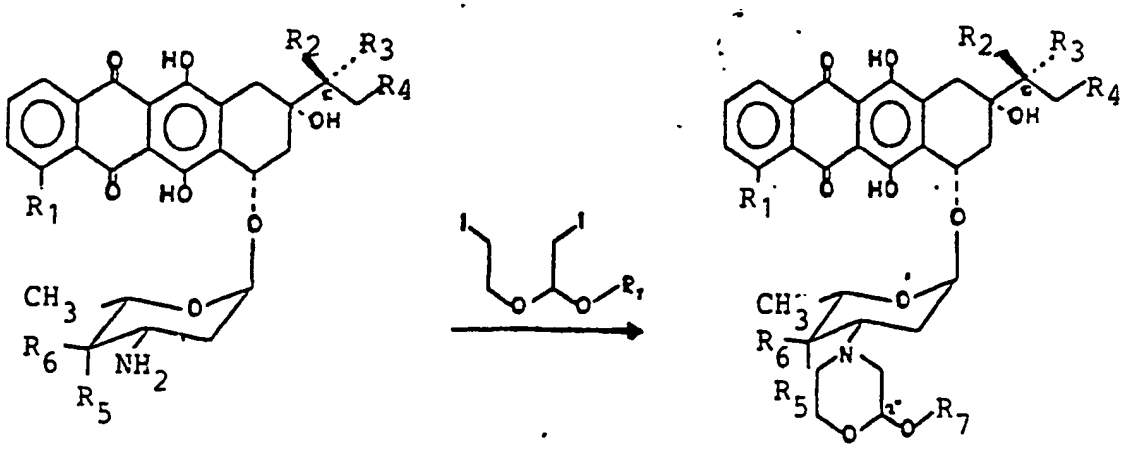
The following reaction Schemes illustrate the processes of the present invention.

Scheme 1



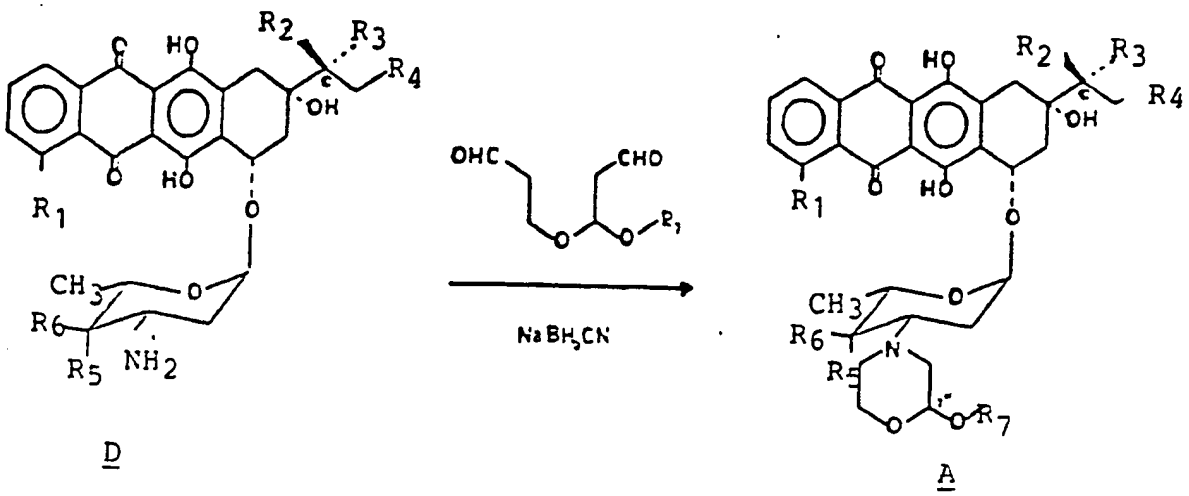
B A

Scheme 2



D A

Scheme 3



D A

The present invention further provides a pharmaceutical composition comprising an anthracycline glycoside of formula A or a pharmaceutically acceptable salt thereof, such as the hydrochloride, together with a pharmaceutically acceptable diluent or carrier. Such a composition may comprise conventional carriers and diluents and may be formulated and administered in conventional manner.

The compounds of the invention are useful in methods of treatment of the human or animal body by therapy. They are useful as anti-tumour agents. A therapeutically effective amount is administered to a patient having a tumour to ameliorate or improve the condition of the patient. An amount sufficient to inhibit the growth of the

Biological Assay

13-(R/S)-dihydro-3'-deamino-3'[2(S)-methoxy-4-morpholinyl] doxorubicin (compound A1), was tested "in vitro" against LoVo and LoVo-resistant-doxorubicin (LoVo/DX) cells using a single cell plating technique after 4 hr treatment (Colony assay). The 50% inhibition concentration (IC_{50}) was calculated on concentration-response curves. Compound A1 was tested in comparison with Doxorubicin and 3'-deamino-3'[2(S)-methoxy-4-morpholinyl]doxorubicin. Data are reported in Table 1.

Table 1: Cytotoxicity after 4 hr treatment IC_{50}=ng/ml(1)

Compound	LoVo IC_{50}(ng/ml)	LoVo/DX IC_{50}(ng/ml)	R.I.(2)
A1	28	118	4.2
Doxorubicin	60	2160	36
3'-deamino-3'-[2(S)-methoxy-4-morpholinyl] doxorubicin	16	33	2

(1) IC_{50}= concentration inhibiting 50% colony growth

(2) R.I.= Resistance Index=(IC_{50} LoVo/DX)/(IC_{50}LoVo)

Compound A1 was evaluated "in vivo" against P388 murine Leukemias, sensitive and resistant to Doxorubicin, in comparison with Doxorubicin and 3'-deamino-3'[2(S)-methoxy-4-morpholinyl]doxorubicin. Data are reported in Table 2.

Table 2: Antitumor activity against P388 and P388/DX (Johnson) Leukemias.

Compound	P388[1]		P388/DX[2]	
	Dose[3] (mg/kg)	T/C[4] %	Dose[3] mg/kg	T/C[4] %
A1	1	161	1	133
Doxorubicin	13-16.9	200-225	13-16.9	86-100
3'-deamino-3'-[2(S)-methoxy-4-morpholinyl] doxorubicin	0.09	250	0.09	250

[1] 10^6 cells/mouse (P388 Leukemia) transplanted i.v. in CDF1 mice.
Treatment i.v. on day 1 after inoculation of tumor.

[2] 10^5 cells/mouse (P388/DX, Johnson) transplented i.v. in CDF1 mice.
Treatment on day 1 after inoculation of tumor.

[3] Optimal Dose

[4] Median survival time; % over untreated controls.

The following Examples illustrate the invention.

Example 1

Preparation of 13-(R/S)dihydro-3'(2-methoxy-4-morpholinyl) doxorubicin (A1)

3'-deamino-3'(2-methoxy-4-morpholinyl)doxorubicin.HCl form (B1): R_1=OCH_3, R_4=R_5=OH, R_6=H, R_7=CH_3) (0.15 g, 0.22 mmole) was dissolved in methanol (25 ml), cooled at 0°C and treated with sodium borohydride (20 mg) under stirring. After five minutes, a mixture of acetone (10ml) and acetic acid (2ml) was added. The reaction mixture was diluted with water (50 ml) and extracted twice with methylene chloride. After that, the aqueous solution was brought to pH 7.2 with aqueous hydrogen carbonate and extracted with methylene chloride. The organic phase was washed with water, separated, dried over anhydrous sodium sulphate, filtered and concentrated to small volume under reduced pressure. The title compound A1 (0.12g, yield 80%) was obtained by adding methanolic anhydrous hydrogen chloride followed by ethyl ether precipitation.

TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride/methanol (6/1 by volume) R_f=0.52

FD-MS: m/e 629 (M+)

^{1}H-NMR (200 MHz, $CDCl_3$); δ 1.38, 1.39 (d, J= 6.6H_3, 3H, 5'-

$\underline{CH_3}$); 1.75 (m, 2H, 2'-$\underline{CH_2}$); 2.3-2.0 (m, 8H, $\underline{CH_2}$-N-$\underline{CH_2}$, 3'-$\underline{H}$, 10ax-$\underline{H}$, 8-$\underline{CH_2}$); 3.2-3.4 (m, 1H, 10e-$\underline{H}$); 3.38 (s, 3H, O-CH-O$\underline{CH_3}$); 3.55 (m, 2H, NCH_2-$\underline{CH}$(H)O, 13-$\underline{CH}$); 3.66 (m, 1H, 4'-$\underline{H}$); 3.8-4.1 (m, 4H, 5'-$\underline{H}$, CHOH$\underline{CH_2}$OH, $NCH_2CH(\underline{H})$-O); 4.08 (s, 3H, 4-O$\underline{CH_3}$); 4.48 (m, 1H, O-$\underline{CH}$-OCH_3); 4.58, 4.63 (s, 1H, 9-$\underline{OH}$); 5.28 (m, 1H, 7-$\underline{H}$); 5.55 (1H, 1'$\underline{3H}$); 7.38, (d, J=7.3Hz, 1H, 3-$\underline{H}$); 7.78 (t, J=7.3Hz, 1H, 2-$\underline{H}$); 8.03 (d, J=7.3Hz, 1H, 1-$\underline{H}$); 13.32, 13.24 (s, 1H, 11-$\underline{OH}$); 13.96, 13.97 (s, 1H, 6-$\underline{OH}$).

Example 2

Preparation of 13-(S)dihydro-3'-deamino-3'(2-methoxy-4-morpholinyl)doxorubicin (A2)

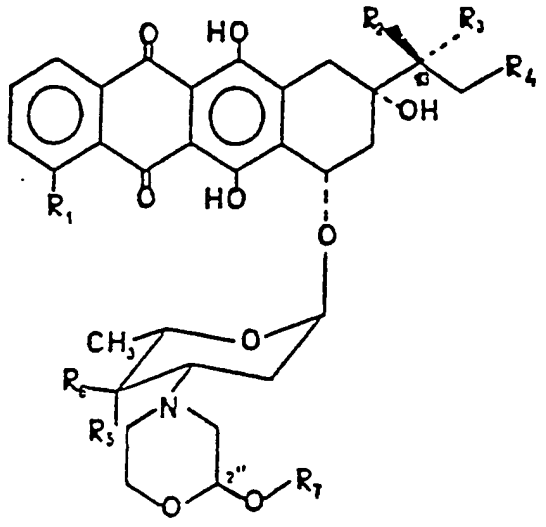
13-(S)-dihydrodoxorubicin.HCl (C1: R_1=OCH_3, R_2=OH and R_3=H, R_4=R_5=OH, R_6=H, R_7=CH_3) (0.10 g, 0.17 mmole) was dissolved in dry dimethylformamide (8 ml) and added with 1,5-diiodo-2(S)methoxyloxy-3-oxa-pentane (D1: X=I, R_7=CH_3) (0.5 g, 2 mmole) and dry triethylamine (0.5 ml, 0.4 mmole). The mixture was kept at room temperature for 24 hours, then was poured into water and extracted with methylene chloride. After standard work-up, the crude product was purified on silicic acid column using as eluting system a mixture of methylene chloride/ methanol (10/1 by volume), to give, after treatment with methanolic anhydrous hydrogen chloride, the title compound A2 (0.04 g, yield 40%).

TLC on Kieselgel Plate F_{254} (Merck), eluting system methylene chloride/methanol (6/1 by volume) R_f=0.52

FD-MS: m/e 629 (M+).

CLAIMS

1. An anthracycline glycoside of general formula A:



A

wherein R_1 is a hydrogen atom, hydroxy or methoxy group; one of R_2 and R_3 represents a hydrogen group and the other of R_2 and R_3 represents a hydroxyl atom; R_4 is hydrogen or hydroxy; both R_5 and R_6 represent hydrogen or one of R_5 and R_6 is hydroxy and the other of R_5 and R_6 represents hydrogen; R_7 represents a lower linear or branched alkyl or benzyl group or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 in which R_7 is an alkyl group containing from 1 to 6 carbon atoms.

3. A compound according to claim 2 which is 13-(R/S)-dihydro-3'-deamino-3'-(2-methoxy-4-morpholinyl)-doxorubicin or its hydrochloride salt.

4. A compound according to claim 2, which is 13-(S)-dihydro-3'-deamino-3'-(2-methoxy-4-morpholinyl)-

doxorubicin or its hydrochloride salt.

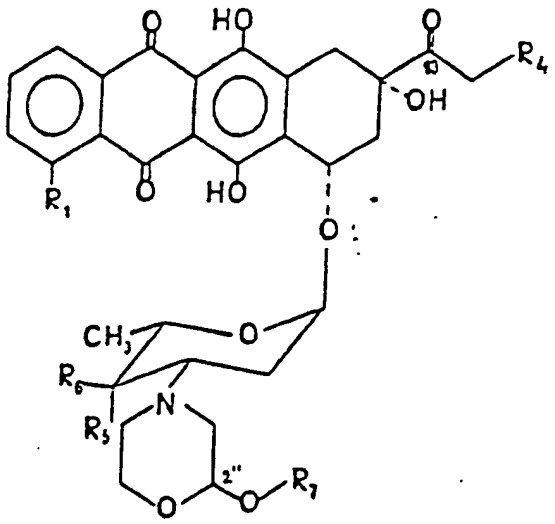
5. A compound according to claim 2, which is 13-(S/R)-dihydro-4'-epi-3'-deamino-3'-(2-methoxy-4-morpholinyl)-doxorubicin or its hydrochloride salt.

6. A compound according to claim 2, which is 13-(S)-dihydro-4'-epi-3'-deamino-3'-(2-methoxy-4-morpholinyl)-doxorubicin or its hydrochloride salt.

7. A compound according to claim 2, which is 13-(S/R)-dihydro-4-demethoxy-3'-deamino-3'-(2-methoxy-4-morpholinyl)-daunorubicin or its hydrochloride salt.

8. A compound according to claim 2, which is 13-(S)-dihydro-4-demethoxy-3'-deamino-3'-(2-methoxy-4-morpholinyl)-daunorubicin or its hydrochloride salt.

9. A process for preparing a compound according to claim 1, which process comprises reducing the 13-carbonyl group of a compound of formula B:



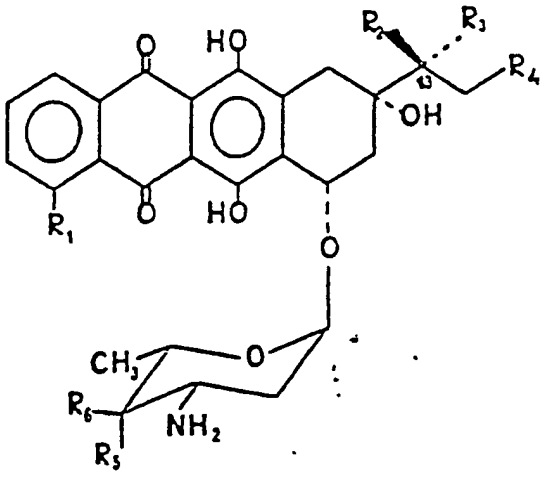
B

wherein R_1, R_4, R_5, R_6 and R_7 are as defined in claim 1, or

a salt thereof and, if desired, converting the resulting compound of formula A into a pharmaceutically acceptable salt.

10. A process according to claim 9, which comprises reacting a compound of formula A, with an alkaline metal borohydride or cyanoborohydride, in an organic solvent, at a temperature of 0°C for five minutes, to give the desired compound as a free base, treating with methanolic hydrogen chloride, and isolating the desired compound as a hydrochloride.

11. A process for preparing a compound according to claim 1, which process comprises reacting an optically pure 13(S)-dihydro anthracycline glycoside of formula D:



D

wherein R_1, R_4, R_5 and R_6 are as defined in claim 1, R_2 represents hydroxyl group and R_3 is hydrogen, or a salt

thereof, with a diiodo or dialdehyde of general formula E

$$X\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH(OR_7)\text{-}CH_2\text{-}X$$

E

wherein X represents an iodine atom or formyl group (-CHO) and R_7 is as defined in claim 1 and, if desired, converting the resulting product of formula A to a pharmaceutically acceptable salt.

12. A process according to claim 11, which is carried out: (a) if X represents iodine in a dry polar aprotic solvent and in the presence of a dry organic base at room temperature from 4 to 24 hours or (b) if X represents formyl, in an aqueous system, at pH from 5 to 4 in presence of a reducing agent and

in which if a free base is obtained it is treated with methanolic hydrogen chloride, to obtain a hydrochloride.

13. A pharmaceutical composition comprising a compound according to claim 1, together with a pharmaceutically acceptable diluent or carrier.

14. A compound as defined in claim 1 for use in a method of treatment of the human or animal body by therapy.

15. A compound according to claim 14 for use as an anti-tumour agent.

16. A process for the preparation of a compound as defined in claim 1, which is substantially as hereinbefore described in any one of the Examples.